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New P Serotype of Group A Human Rotavirus Closely Related to That of a Porcine Rotavirus

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The VP7 and VP4 genes of two human group A rotavirus strains Mc323 and Mc345 with unique serologic and genomic properties, and isolated in Chiang Mai, Thailand, in 1989 [Urasawa et al. (1992) Journal of Infectious Diseases 166:227–234] were further characterized. The nucleotide and deduced amino acid sequences of the VP7 genes allowed the classification of both strains as serotype G9. The VP4 genes of both strains are 2,359 nucleotides in length and encode a protein of 775 amino acids like in most human rotaviruses. A comparison of the VP4 amino acid sequence of strain Mc323 with those of strain Mc345 and 24 human and animal rotaviruses representing 20 distinct VP4 genotypes reported to date showed that VP4 of Mc323 and Mc345 belong to genotype 19 previously reported for porcine rotavirus [Burke et al. (1994) Journal of General Virology 75:2205–2212]. To investigate the serological type (P serotype) of these VP4s, six reassortant viruses each containing a distinct VP4 gene characteristic of human rotaviruses and the VP7 gene of porcine rotavirus strain Gottfried (G4) were prepared, and antisera to these reassortants produced in rabbits. In neutralization tests, the P serotype of Mc323 was clearly differentiated from the five major P serotypes reported previously for human rotaviruses, suggesting that Mc323 and Mc345 represent a new human rotavirus P serotype tentatively called P11. ***J. Med. Virol. 60:63–69, 2000.***

KEY WORDS: nucleotide sequence; VP7 gene; VP4 gene; P serotype; VP4 genotype

INTRODUCTION

A rotavirus particle consists of 3 protein layers (outer capsid, inner capsid, and core) and 11 viral RNA segments enclosed within them. The major core protein is VP2 enclosing the genome with VP1 and VP3 proteins attached. The major inner capsid protein of group A rotavirus is VP6, encoded by RNA segment 6, on which group-specific and subgroup-specific antigens are located. The outer capsid of group A rotavirus consists of two structural proteins, VP4 and VP7. VP4 encoded by RNA segment 4 is responsible for several important biological functions including protease-enhanced infectivity, serotype-specific antigenicity, interaction with cellular receptor, fusion activity, plaque formation, and virulence. VP7, a glycoprotein representing the main outer capsid protein and cell attachment protein and carrying serotype-specific neutralization epitopes, is encoded by segment 7, 8, or 9, depending on strain [Estes, 1996].

The specificities of the neutralization antigens on VP7 and VP4 have been designated as the G serotype (for *g*lycoprotein) and the P serotype (for *p*rotease-sensitive protein), respectively. Fourteen different G serotypes have been described [Estes, 1996]. Of these, eight (G1–G4, G5, G8, G9, and G12) have been found in human rotaviruses, whereas another two, G6 and G10, isolated from diarrheic children in Italy and Thailand, respectively, were found to be more closely related genomically to bovine than to human rotaviruses [Gerna et al., 1992; Urasawa et al., 1992, 1993; Gouvea et al., 1994; Estes, 1996]. In contrast, P serotypes have not yet been fully analyzed, as VP4 antigen in rotavirus virion is poorly immunogenic compared with VP7 antigen upon immunization of animals.

Two different classification systems of VP4 have been proposed: one is defined by VP4 sequence analysis or RNA-RNA hybridization [Estes, 1996] and is termed the VP4 genotype [Sereno and Gorziglia, 1994], and the other is determined by serologic analysis based on neutralization test using antisera prepared against baculovirus-expressed VP4 proteins or reassortants with particular VP4 genes [Gorziglia et al., 1990; Snodgrass et al., 1992] and is termed the P serotype [Sereno and Gorziglia, 1994]. At least 20 different VP4 genotypes

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with 9 in humans (3, 4, 6, 8, 9, 10, 11, 12, and 14) have been identified to date, whereas 13 different P serotypes (including subtypes) with 8 in humans (1A, 1B, 2A, 3A, 3B, 4, 5, and 8) have been reported [Sereno and Gorziglia, 1994; Mphahlele and Steele, 1995; Estes, 1996]. VP4 genotypes and P serotypes have been correlated to the effect that for a number of serotypes the genotypes have been identified but there are still genotypes that have no serotype equivalent name. The current nomenclature takes this into account by naming a dually identified type, e.g., P1A[8], whereas a type identified as genotype alone is just given a number in square brackets, e.g., P[15] [Estes, 1996].

In the United States, a license has been granted to a tetravalent (G1–G4) rotavirus reassortant vaccine in August 1998 [ACIP (1999) MMWR 48, Vol RR-2, 1–22] and may also be introduced into other countries in the near future. Therefore, knowledge of virus neutralization antigens VP7 and VP4 of strains prevalent in different countries is important not only from an epidemiologic standpoint but in view of the potential implications for vaccine control strategies.

In our previous study on the distributions of subgroup, serotype, and RNA electropherotype of group A rotaviruses collected in Thailand, two human rotaviruses with unusual antigenic and genomic properties were isolated [Urasawa et al., 1992]. Strain Mc323 had subgroup I and presumably G serotype 9 antigens and a long RNA pattern, while strain Mc345 had the same subgroup and serotype antigens and an anomalous RNA pattern that was characterized by the presence of an aberrant segment migrating between segments 6 and 7 and no segment 11. Analysis by RNA-RNA hybridization showed that the two strains are genetically very closely related to each other and that they are more related to porcine than to human rotaviruses [Urasawa et al., 1992]. Our later studies [Kojima et al., 1996] disclosed that the aberrant gene migrating between segments 6 and 7 of Mc345 is a rearranged NSP5 gene (usually segment 11) and that the NSP1 sequence of both strains is more related to that of porcine than to human rotaviruses.

In the present study, we sequenced the VP7 and VP4 genes of strains Mc323 and Mc345 and compared their nucleotide and deduced amino acid sequences with those of other established human and animal rotaviruses. Further, the P serotypes of these strains were investigated in neutralization tests using viruses with different VP4 genes reported for human rotaviruses and antisera against reassortants thereof prepared for this investigation.

MATERIALS AND METHODS

Viruses

Strains Mc323 and Mc345 were obtained in Chiang Mai, Thailand, and isolated in MA104 cells as described previously [Urasawa et al., 1992]. Human rotavirus strains representing distinct P serotypes were employed in neutralization reaction: KU (G1, P1A[8]), S2 (G2, P1B[4]), M37 (G1, P2A[6]), K8 (G1, P3A[9]), 69M (G8, P4[10]), Mc35 (G10, P3B[14]). These viruses were propagated in MA104 cells in the presence of 1 μg/ml acetylated trysin (type V-S from bovine pancreas; Sigma, St. Louis, MO).

Nucleotide Sequence Determination

Single-shelled particles were purified from virus-infected culture fluid by differential centrifugation, fluorocarbon treatment, treatment with EDTA, and CsCl density gradient centrifugation. Single-stranded (ss) RNA was synthesized in vitro by utilizing the endogenous RNA-dependent RNA polymerase (transcriptase) present in single-shelled particles [Cohen, 1977] and was precipitated in 2 M LiCl. The nucleotide sequences of the VP7 and VP4 genes of strains Mc323 and Mc345 were determined by using dideoxynucleotide sequencing reactions with a series of synthetic oligonucleotide primers (17- to 25-mers), reverse transcriptase from avian myeloblastosis virus (Seikagaku Kogyo, Tokyo, Japan), and [^{32}P]dATP (3,000 Ci/mmol; Amersham, Arlington Heights, IL) as described previously [Gorziglia et al., 1986; Taniguchi et al., 1994]. The 200 3′-terminal nucleotides of the RNA were determined by using denatured double-stranded (ds) RNA in 50% dimethyl sulfoxide after boiling for 2 min instead of ssRNA transcript.

Preparation of Rotavirus Reassortants

The human rotaviruses selected to introduce VP4 genes (segment 4) into reassortants were Wa (G1, P1A[8]), DS-1 (G2, P1B[4]), M37 (G1, P2A[6]), K8 (G1, P3A[9]), 69M (G8, P4[10]), and Mc323 (G9, P unknown). The porcine rotavirus strain Gottfried (G4, P2B[6]) was used as a donor for VP7 gene (segment 9), since its growth capability was similar to that of the human viruses cited above and since the migrating rate of its genome segment 4 in polyacrylamide gel electrophoresis (PAGE) was different from that of human viruses in PAGE, allowing the determination of VP4 gene (segment 4) origin. Reassortant viruses were generated by coinfection of MA104 cells with trypsin-treated parental strains (at a multiplicity of infection of about 3), occasionally in the presence of monoclonal antibodies specific to VP7 of the human parent. Reassortants with P1A and P2A VP4 genes were generated by coinfection with strains Wa and M37, respectively, and a reassortant with P3A VP4 gene derived from strain K8. Plaques of progeny viruses were propagated in MA104 cells and the migration of RNA segments was examined in PAGE. The finally selected reassortants were plaque-purified three times. All the reassortants contained segment 9 (VP7 gene) from Gottfried and segment 4 from the human parent. The Gottfried origin of VP7 gene (encoding G4) in all the reassortants was confirmed by ELISA with G serotype-specific monoclonal antibodies (manuscript in preparation). The reassortants obtained were labeled as follows: Go-Wa (G4, P1A[8]), Go-DS-1 (G4, P1B[4]), Go-M37 (G4, P2A[6]), Go-K8 (G4, P3A[9]), Go-69M (G4, P4[10]), and

TABLE I. Genomic Composition of Reassortant Viruses*

	RNA segment										
Reassortant	1	2	3	4	5	6	7	8	9	10	11
Go-Wa (G4, P1A[8])	—	K8	K8	**Wa**	K8	K8	Go	Go	**Go**	K8	Go
Go-DS-1 (G4, P1B[4])	DS1	DS1	DS1	**DS1**	DS1	Go	DS1	Go_7	**Go**	Go	Go
Go-K8 (G4, P3A[9])	—	K8	K8	**K8**	K8	K8	Go	Go	**Go**	K8	Go
Go-M37 (G4, P2A[6])	—	K8	K8	**M37**	K8	—	Go	Go	**Go**	K8	—
Go-69M (G4, P4[10])	69M	69M	69M	**69M**	69M	69M	Go	—	**Go**	69M	69M
Go-Mc323 (G4, P?)	Mc	Mc	Mc	**Mc**	Mc	Mc	Go	Mc_7	**Go**	Mc	Mc

*VP4 and VP7 gene origin are shown in boldface. Go, Gottfried; Mc, Mc323; —, segment origin undetermined; Go_7, segment 7 of Gottfried; Mc_7, segment 7 of Mc323.

Go-Mc323 (G4, P?). Genomic composition of the reassortants known from PAGE analysis is shown in Table I.

Production of Antisera

Polyclonal antisera to the reassortants were prepared by intravenously injecting concentrated virus into weanling rabbits two or three times at 2-week intervals. Each rabbit was reared in a separate room during immunization. Rabbits were tested for rotavirus antibodies before immunization.

Serological Assays

Neutralizing antibody titers were determined by microtiter fluorescent focus reduction tests using 200 focus forming units of virus, with endpoint titers determined as 60% reduction of foci [Pongsuwanna et al., 1996].

Nucleotide Sequence Accession Number

Sequence data from the present study have been deposited with the GSDB, DDBJ, EMBL, and GenBank data libraries under accession numbers D38052 (strain Mc323 VP4 gene), D38053 (strain Mc323 VP7 gene), D38054 (strain Mc345 VP4 gene), and D38055 (strain Mc345 VP7 gene).

RESULTS

Nucleotide Sequence of VP7 Gene of Mc323 and Mc345

The structure of the VP7 genes of strains Mc323 and Mc345 was similar to those of other human rotaviruses. The VP7 genes of both strains are 1,061 base pairs in length, with a single open reading frame (ORF) initiating (ATG) at positions 49 to 51 and terminating (TGA) at positions 1027 to 1029. Unlike human rotaviruses of the other types, the length of the 3′-noncoding regions is 35, with one base deletion just downstream of the termination codon as found in other representative G9 human rotavirus strains WI61 and F45. Both ORFs have the capacity to encode a VP7 protein of 326 amino acids. The nucleotide sequence of the Mc323 VP7 gene and the deduced amino acid sequence were compared with those of Mc345 and 17 strains representing serotypes G1–G14 reported so far (data not shown). The homology in the VP7 gene between strain Mc323 and strains of all G serotypes except G9 was only 65.1–77.7% and 58.3–84.4% in nucleotide and amino acid sequences, respectively. By contrast, a high degree of nucleotide and amino acid sequence homologies was observed between Mc323 and two representative G9 strains, WI61 (89.7% and 95.4%) and F45 (89.2% and 95.1%), and between Mc323 and Mc345 (96.9% and 95.1%). It was of note that although strains Mc323 and Mc345 were isolated in the same year and place, the homologies of Mc345 to WI61 (88.3% and 92.3%) and F45 (88.0% and 92.0%) were slightly lower than those of Mc323 mentioned above. Amino acid substitutions between the two Thai strains were found over the entire VP7 protein except for the initial (1–45 amino acids) and terminal (240–326 amino acids) portions and not restricted to variable regions VP1–VP9 [Kapikian and Chanock, 1996]. These results, together with their property peculiar to G9 strains (i.e., one base deletion just after the termination codon), confirmed assignment of the two Thai strains to serotype G9, which was previously suggested by cross-neutralization tests with serotype-specific antisera [Urasawa et al., 1992].

Nucleotide Sequence of VP4 Gene of Mc323 and Mc345

The entire VP4 gene of both Mc323 and Mc345 was found to be 2,359 base pairs in length, with 5′- and 3′-noncoding regions of 9 and 25 nucleotides, respectively. The gene contains a single long ORF of 2,325 base pairs, capable of encoding a protein of 775 amino acids, like most human rotaviruses except for strain 69M (776 amino acids) (Fig. 1). VP4 genes of the majority of animal rotaviruses, in contrast, encode 776 amino acids.

Comparative sequence analysis of VP4 genes of various human and animal rotaviruses (data not shown) indicated that four cysteine residues at positions 215, 317, 379, and 773, observed in almost all human and animal rotaviruses, are also conserved in VP4 of strains Mc323 and Mc345. Although two potential trypsin cleavage sites (arginine in amino acid positions 240 and 246) are present in both strains as in most human and animal rotaviruses (for the latter in positions 241 and 247), the sequence of the connecting peptide of the two isolates that are identical to each other are considerably different from those of other strains. Two amino acid residues corresponding to positions 136 and 189 in animal rotaviruses are missing in both strains, and one amino acid residue (serine) corre-

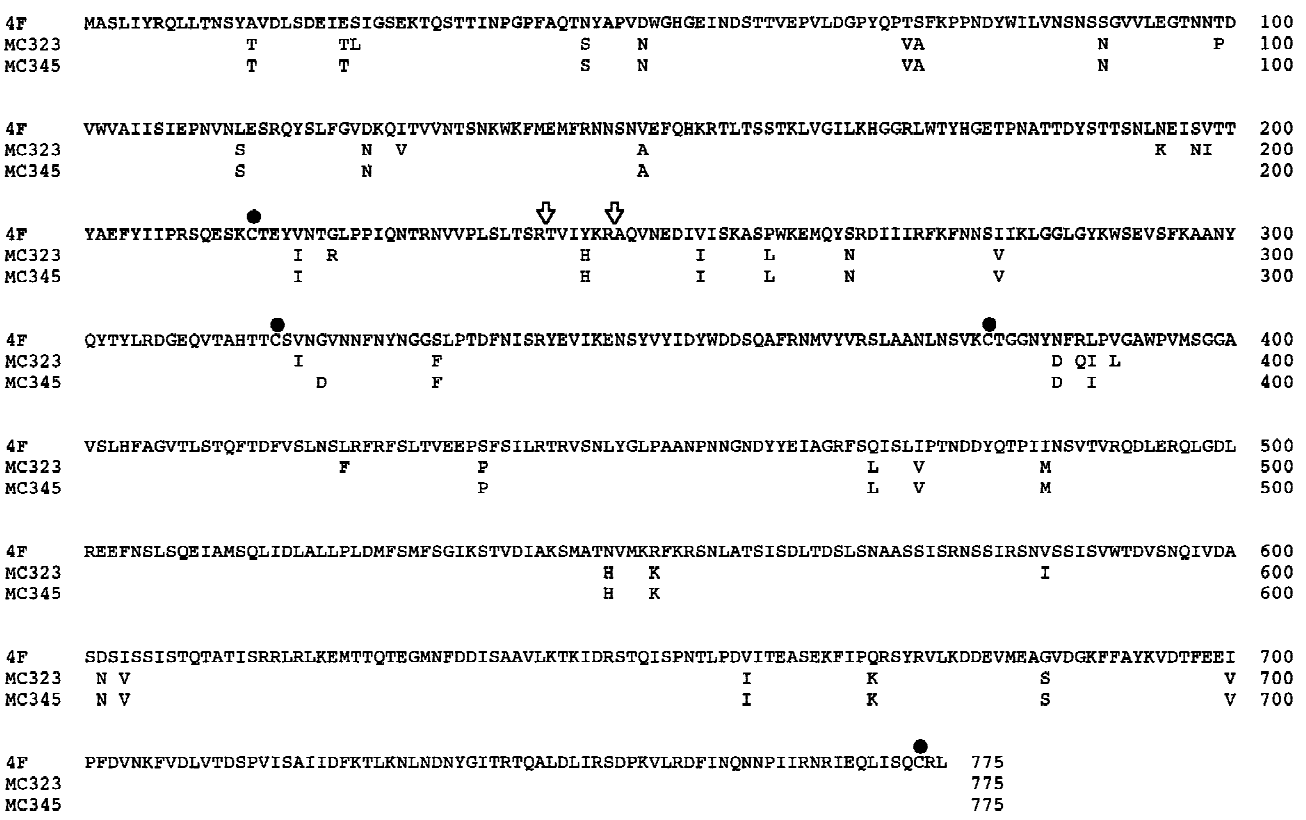

Fig. 1. Comparison of VP4 amino acid sequences of a porcine rotavirus 4F representing genotype 19 and human rotavirus strains Mc323 and Mc345. The amino acid sequence of the 4F VP4 is shown in full, and only the differences from 4F are indicated for the other strains. The entire VP4 amino acid sequence of 4F was previously described [Burke et al., 1994]. The positions of the four conserved cysteines in 4F are shown by dots. The protease cleavage sites are shown by arrows.

sponding to position 188 in the two strains as well as in most human rotaviruses is missing in almost all animal rotaviruses. In addition, a cysteine residue characteristic to animal rotavirus at position 203 (position 202 in human rotavirus) is absent in both strains. These results suggested that the VP4 gene of these strains may be derived from human rotaviruses.

Table II shows the comparison between the nucleotide and deduced amino acid sequences of Mc323 VP4 and those of various human and animal rotavirus VP4s reported previously. Sereno and Gorziglia [1994] described that rotaviruses showing a VP4 amino acid homology of 89% or greater belonged to the same VP4 genotype. Based on this criterion, new VP4 genotypes have been assigned to recently isolated strains whose VP4 sequences were identified. The numbering of VP4 genotypes in Table II follows that described by Sereno and Gorziglia [1994], Taniguchi et al. [1994], and Estes [1996]. The nucleotide and amino acid sequences of Mc323 VP4 showed 63.8–77.7% and 57.0–83.5% identity, respectively, with the hitherto reported VP4 genotypes except P[19]. In contrast, Mc323 VP4 was highly homologous to genotype P[19] VP4 of porcine rotavirus 4F (89.4% in nucleotide and 94.5% in amino acid sequence) as well as to Mc345 VP4 (97.7% and 98.3%).

It was further demonstrated that the degree of VP4 amino acid homology between the different VP4 genotypes declines in the order of VP4, VP8 (a trypsin cleavage product of VP4 containing the major P serotype antigenic site(s)), and region B (a P serotype-specific region of VP8 spanning amino acids 92–192) [Larralde and Gorziglia, 1992; Kapikian and Chanock, 1996]. Therefore, the amino acid sequence of the VP8 subunit and the B region of Mc323 VP4 was compared with those of the same human and animal rotavirus strains described above (Table II). Except for 4F and Mc345, the sequence homologies were 32.5–72.8% in the VP8 subunit and 11.9–68.3% in the B region. By contrast, extremely high homologies were found between Mc323 and 4F (92.3% in the VP8 and 95.1% in the B region) and between Mc323 and Mc345 (97.2% in the VP8 and 98.0% in the B region). From these results we conclude that strains Mc323 and Mc345 share the same VP4 genotype and are assigned to genotype P [19].

Reactions of Antisera to Reassortants With Parental Human Virus

Pre-immunization sera of weanling rabbits employed possessed neutralizing activity ranging from <32 to 256. Antisera to each of the six reassortants reacted to high titers with parental porcine virus Gottfried possibly due to its common VP7 (G4) antigen in all these reassortants (Table III). These antisera reacted with the respective parental human viruses at titers equal

TABLE II. Comparison of Sequence Identity of VP4 of Strain Mc323 With Various Human and Animal Rotaviruses*

Strain (origin)	VP4 genotype	P serotype	% Identity of Mc323 VP4 with indicated strain in: VP4 nucleotide sequence	Amino acid sequence: VP4	VP8	B region[a]
NCDV (bovine)	1	6	71.6	74.7	61.8	49.5
SA11 (simian)	2		73.2	76.1	63.8	46.5
RRV (simian)	3	5	71.4	76.0	61.0	45.5
HCR3 (human)	3	5	72.6	75.4	61.0	46.5
K9 (canine)	3	5	72.6	76.3	63.5	46.5
DS1 (human)	4	1B	76.2	79.1	71.5	59.4
UK (bovine)	5	7	69.0	70.7	58.1	50.1
M37 (human)	6	2A	76.6	83.5	70.0	68.3
1076 (human)	6	2A	76.3	81.7	69.9	66.3
Gottfried (porcine)	6	2B	76.2	83.0	71.5	67.3
OSU (porcine)	7	9	72.4	73.4	58.9	41.6
KU (human)	8	1A	77.7	80.4	72.8	63.4
K8 (human)	9	3A	66.7	65.6	50.8	31.7
69M (human)	10	4	72.2	75.1	61.5	49.5
KK3 (bovine)	11	8	63.8	57.0	32.5	11.9
FI23 (equine)	12		73.1	74.7	61.8	50.5
MDR13 (porcine)	13		72.0	72.1	56.5	42.6
Mc35 (human)	14	3B	66.2	65.3	52.0	29.7
Lp14 (ovine)	15		73.5	76.0	62.6	45.5
Eb (murine)	16	10	67.5	69.3	54.5	39.6
993/83 (bovine)	17		64.4	58.3	33.7	21.8
L338 (equine)	18		72.2	72.3	59.8	48.5
4F (porcine)	**19**		**89.4**	**94.5**	**92.3**	**95.1**
EHP (murine)	20		68.9	73.9	60.6	48.5
Mc345 (human)	**(19)**		**97.7**	**98.3**	**97.2**	**98.0**

*GenBank accession nos. of VP4 genes: NCDV (C31159); SA11 (D16347); RRV (M18736); HCR3 (L19712); K9 (D14725); DS1 (P11196); UK (P12474); M37 (L20877); 1076 (M88480); Gottfried (M33516); OSU (X13190); KU (M21014); K8 (D90260); 69M (M60600); KK3 (D13393); FI23 (D16342); MDR13 (L07887); Mc35 (D14032); Lp14 (L11599); Eb (L18992); 993/83 (D16352); L338 (D13399); 4F (L29184); EHP (V08424); Mc345 (D38054).
[a]B region spanning aa 92–192.

TABLE III. Neutralization Reactions of Parental Human Rotaviruses With Antiserum to Reassortants*

Virus (G and P serotypes)	Titer with antiserum to reassortant: Go-Wa (G4, P1A[8])	Go-DS-1 (G4, P1B[4])	Go-M37 (G4, P2A[6])	Go-K8 (G4, P3A[9])	Go-69M (G4, P4[10])	Go-Mc323 (G4, P?)
KU (G1, P1A[8])	8,192	512	128	256	256	512
S2 (G2, P1B[4])	2,048	8,192	512	512	512	1,024
M37 (G1, P2A[6])	<512	256	4,096	256	128	<512
K8 (G1, P3A[9])	<512	<256	<128	4,096	256	<512
Mc35 (G10, P3B[14])	2,048	1,024	128	1,024	512	<512
69M (G8, P4[10])	<512	512	128	128	4,096	<512
Mc323 (G9, P?)	1,024	1,024	256	256	512	16,384
Gottfried (G4, P2B[6])	32,768	16,384	8,192	8,192	8,192	16,384

*Results are expressed as the reciprocal of neutralization titers by 60% fluorescent focus reduction tests. Titers between reassortant virus and parental human virus are underlined.

to or 2- to 4-fold lower than with Gottfried. However, antisera to the reassortants could distinguish between parental human viruses representing distinct P serotypes by more than 8-fold difference in titer except for three reactions, anti-Go-Wa vs S2, anti-Go-K8 vs Mc35, and anti-Go-Wa vs Mc35, in which only 4-fold difference was observed. While the former two reactions might be due to the antigenic relatedness between subtypes 1A and 1B and 3A and 3B, respectively, the reason for the latter is unknown. In contrast, Mc323 was neutralized by the reassortant antisera at titers 8-fold or more lower than the homologous titers, whereas Go-Mc323 reassortant antiserum did not react significantly with six human viruses with distinct P serotype (titers >16-fold less than with VP4 parent virus). These results strongly suggest that Mc323 represents a new P serotype that is tentatively called P11, completing the nomenclature to P11[19].

DISCUSSION

Sequencing of VP7 gene of strains Mc323 and Mc345 in this study led to the conclusion that the two strains that had previously been serologically classified as G9 [Urasawa et al., 1992] can be confirmed as G9 on the basis of sequence analysis.

VP4 gene of the two strains was also sequenced. The results showed that all the characteristics observed in the VP4 genes of Mc323 and Mc345 were shared by that of porcine 4F variant representing genotype P[19] (Fig. 1). Porcine rotavirus variants 4S and 4F, differing

in in vitro growth properties and pathogenicity, had been isolated from the feces of a diarrheic pig in China by Burke et al. [1994]. 4S and 4F had VP4 genes of genotype P[5] and of a novel genotype now identified as P[19], respectively, while they possessed VP7, NS53, and VP6 genes, which were almost identical to each other. These results, together with our previous finding showing genetic relatedness of the Thai human strains closer to porcine than to human rotaviruses in RNA-RNA hybridization test and in sequence analysis of NSP1 gene, strongly suggest the porcine origin of several gene segments of the Thai strains, although the origin of their VP4 genes remained undetermined since they possessed some characteristics in nucleotide sequence common to human rotavirus VP4 gene while sharing P[19] genotype with porcine rotavirus.

Many researchers have reported a close genetic relationship between human and animal rotaviruses [Nakagomi and Nakagomi, 1989; Nakagomi et al., 1990b; Gerna et al., 1992; Urasawa et al., 1992; Das et al., 1993]. These results strongly support the transmission of rotaviruses between humans and animals; such strains transmitted across the species barrier might very rarely become adapted to a perpetual growth in the new host, or a reassortant virus occurring as a result of simultaneous infection of the host with the endogenous strain and the transmitted strain might acquire the ability for a perpetual growth.

The determination of rotavirus P serotype was first made successfully by Gorziglia et al. by neutralization reaction with P serotype-specific antisera prepared against baculovirus-expressed VP4 proteins [Gorziglia et al., 1990; Sereno and Gorziglia, 1994], but it has not been widely put into practice due to the difficulty in preparing these antisera and their limited number and volume available. On the other hand, P serotyping by neutralization reaction using antisera prepared against reassortants with distinct VP4 genes has been carried out for a limited number of animal rotaviruses [Snodgrass et al., 1992; Isa and Snodgrass, 1994]. In this study, therefore, the P serotype of Mc323, having a unique human rotavirus VP4, was examined using rabbit antisera prepared by immunization against reassortants carrying porcine rotavirus VP7 (G4) and VP4 of major human rotavirus P serotypes except for P5 and P8. In this experiment, the specificity of the VP4-specific antisera was found to be relatively low. This might be due to antibodies produced against cross-reactive epitopes held in common among distinct human rotavirus VP4s. Or, more likely, this may be due to cross-reactive antibodies from previous rabbit rotavirus infections as suggested by the presence of neutralizing activity in pre-immunization sera. Unexpectedly, a weak cross-reactivity was observed between P1A (Go-Wa) antiserum and P3B (Mc35) serotype antigen. The phenomenon reported by Chen et al. [1992] that the serologic reactivity of defined VP4s can be altered if VP4 is located as a neighbor of VP7 of heterologous parental origin in reassortants might give an explanation for this result. The results of cross-neutralization reactions, especially those of antiserum to the reassortant Go-Mc323, however, strongly suggest that Mc323 VP4 represents a P serotype clearly distinguishable from the human rotavirus P serotypes reported to date. It is tentatively called P11.

As for G9 strains, G9-P1A[8] strains with subgroup II antigen and long RNA electropherotype were detected previously several times in Japan [Nakagomi et al., 1988; Nakagomi et al., 1990a]. Recently, it has been reported that G9-P2A[4] strains (subgroup I, short RNA electropherotype) and G9-P2A[4] strains (subgroup II, long RNA electropherotype) were highly prevalent in India [Ramachandran et al., 1996], Bangladesh, and the United States [Ramachandran et al., 1998]. Here we reported on G9 strains isolated in Chiang Mai, Thailand, having subgroup I antigen, long RNA electropherotype, and a new P serotype antigen (VP4 genotype 19) hitherto not found in human rotavirus. This is tentatively called P11[19]. At least two more G9 human strains with subgroup I antigen and long RNA electropherotype have been found in Bangkok, Thailand, in 1991–92 [Pongsuwanna, 1995], and their VP4 gene sequence is now being investigated.

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